FIG.1A. NUCLEOTIDE SEQUENCE OF THE PIV-3 F GENE (PCR-AMPLIFIED)

```
AAGTCAATACCAACAACTATTAGCAGTCATACGTGCAAGAACAAGAAGAGATTCAA
TTCAGTTATGGTTGTTGATAATCGTCAGTAGCACGTTCTTGTTCTTCTTCTTAAGTT
        10        20        30        40        50        60

AAAGCTAAATAAGAGAAATCAAAACAAAGGTATAGAACACCCGACCACAAATCAAAA
TTTCGATTTATTCTCTTTAGTTTTGTTTCCATATCTTGTTTGTTTTTAGTTTT
        70        80        90       100       110       120

CATCCAATCCATTTAAACAAAAATTCCAAAAGAGACCGGCAACAACAAGCACCAAAC
GTAGGTTAGGTAAAATTTGTTTTTAGGTTTTCTTGGCCGTTGTTGTTCGTGGGTTTG
       130       140       150       160       170       180
                           SP

         MET PRO THR [LEU] ILE LEU LEU ILE ILE THR THR MET ILE MET ALA [SER] SER CYS GLN
ACAATGCCAACTTTAATTACTGCTAATTATTACAACAATGATTATTGGCATCTTCCTGCCAA
TGTTACGGTTGAAATTATGACGATTAATAATGTTGTTACTAATACCGTAGAAGGACGGTT
       190       200       210       220       230       240

ILE ASP ILE THR LYS LEU GLN HIS VAL GLY VAL LEU VAL ASN SER PRO LYS GLY MET LYS
ATACATATCACAAAACTACAACAGCATGGTAGGTGTATTGGTCAACAGTCCCAAAGGGATGAAG
TATGTATAGTGTTTTGATGTCGTACATCCACATAACCAGTTGTCAGGTTCCCTACTTC
       250       260       270       280       290       300

ILE SER GLN ASN PHE GLU THR ARG TYR LEU ILE LEU SER LEU ILE PRO LYS ILE GLU ASP
ATATCACAAAACTTCGAAACAAGATATCTAATTTTGAGCCTCATACCAAAAATAGAAGAC
TATAGTGTTTTGAAGCTTTGTTCTATAGATTAAAACTCGGAGTATGGTTTTATCTTCTG
       310       320       330       340       350       360

SER ASN SER CYS GLY ASP GLN GLN ILE LYS GLN TYR LYS ARG LEU LEU ASP ARG LEU ILE
TCTAACTCTTGTGGTGACCAACAGCAGATCAAACAATACAAGAGGTTATTGGATGACTGATC
AGATTGAGAACACCACTGGTTGTTCTAGTTTGTTATGTTCCAATAACCTATCTGACTAG
       370       380       390       400       410       420
```

FIG.1B.

ILE PRO LEU TYR ASP GLY LEU ARG LEU GLN LYS ASP VAL VAL ILE VAL [THR] ASN GLN GLU SER
```
ATCCCTCTATATGATGGATTAAGATTACAGAAAGATGTGATAGTAGTAACCAATCAAGAATCC
TAGGGAGATATACTACCTAATTCTAATGTCTTTCTACACTATCATCATTGGTTAGTTCTTAGG
    430       440       450       460       470       480
```

ASN GLU ASN THR ASP PRO ARG THR [ARG] ARG [SER] PHE GLY GLY VAL ILE GLY THR ILE ALA
F2-F1 CLEAVAGE SITE
```
AATGAAAACACTGATCCCAGAACAAGACGATCCTTTGGAGGGTAATTGGAACCATTGCT
TTACTTTTGTGACTAGGGTCTTGTTCTGCTAGGAAACCTCCCATTAACCTTGGTAACGA
    490       500       510       520       530       540
```

LEU GLY VAL ALA THR SER ALA GLN ILE THR ALA ALA VAL ALA LEU VAL GLU ALA LYS GLN
```
CTGGGAGTAGCAACCTCAGCACAATTACAGCGGCAGTTGCTCTGGTTGAAGCCAAGCAG
GACCCTCATCGTTGGAGTCGTGTTAATGTCGCCGTCAACGAGACCAACTTCGGTTCGTC
    550       560       570       580       590       600
```

ALA [LYS] SER ASP ILE GLU LYS LEU LYS GLU ALA ILE ARG ASP THR ASN LYS ALA VAL GLN
```
GCAAAATCACACATCGAAAAACTCAAAGAAGCAATCAGGGACACAAACAAAGCAGTGCAG
CGTTTAGTGTGTAGCTTTTTGAGTTCTTCGTTAGTCCCTGTGTTTGTTTCGTTCACGTC
    610       620       630       640       650       660
```

SER VAL GLN SER SER ILE GLY ASN LEU ILE VAL ALA ILE LYS SER VAL GLN ASP TYR VAL
```
TCAGTTCAGAGCTCTATAGGAAATTAATAGTAGCAATTAAATCAGTCAGGATTATGTC
AGTCAAGTCTCGAGATATCCTTTAAATTACATCGTTAATTTAGTCAGGTTCTAATACAG
    670       680       690       700       710       720
```

ASN [ASN] GLU ILE VAL PRO SER ILE ALA ARG LEU GLY CYS GLU ALA ALA GLY LEU GLN LEU
```
AACAACGAAATCGTGCCATCGATTGCTAGACTAGGTTGTGAAGCAGCAGGACTTCAATTA
TTGTTGCTTTAGCACGGTAGCTAACGATCTGATCCAACACTTCGTCGTCCTGAAGTTAAT
    730       740       750       760       770       780
```

GLY ILE ALA LEU THR GLN HIS TYR SER GLU LEU THR ASN ILE PHE GLY ASP ASN ILE GLY
```
GGAATTGCATTAACACAGCATTACTCAGAATTAAACAAACATATTGGTGTAACATAGGA
CCTTAACGTAATTGTCGTAATGAGTCTTAATTGTTTGTATAAACCACCATTGTATCCT
    790       800       810       820       830       840
```

FIG.1C.

SER LEU GLN GLU LYS GLY ILE LYS LEU GLN GLY ILE ALA SER LEU TYR ARG THR ASN ILE
TCGTTACAAGAAAAAGGAATAAAATTACAAGGTATAGCATCATTATACCGCACAAATATC
AGCAATGTTCTTTTTTCCTTATTTTAATGTTCCATATCGTAGTAATATGGCGTGTTTATAG
850 860 870 880 890 900

THR GLU ILE PHE THR THR SER THR VAL ASP LYS TYR ASP ILE TYR ASP LEU LEU PHE THR
ACAGAAATATTCACAACATCAACAGTTGATAAATATGATATCTATGATCTATTACA
TGTCTTTATAAGTGTTGTAGTTGTCAACTATTTATACTATAGATACTAGATAATAAATGT
910 920 930 940 950 960

GLU SER ILE LYS VAL ARG VAL ILE ASP VAL ASP LEU ASN ASP TYR SER ILE THR LEU GLN
GAATCAATAAAGGTGAGAGTTATAGGTTGATTGAATGATTACTCAATCAATCACCCTCCAA
CTTAGTTATTTCCACTCTCAATATCTCAATATCTACAACTAAACTTACTAATGAGTTGGGAGGTT
970 980 990 1000 1010 1020

VAL ARG LEU PRO LEU LEU THR ARG LEU LEU ASN THR GLN ILE TYR [LYS] VAL ASP SER ILE
GTCAGACTCCCTTATTAACTAGGCTGCTGAACACTCAGATCTACAAAGTAGATTCCATA
CAGTCTGAGGGAAATAATTGATCCGACGACTTGTGAGTCTAGATGTTCATCTAAGGTAT
1030 1040 1050 1060 1070 1080

SER TYR ASN ILE GLN ASN ARG GLU TRP TYR ILE PRO LEU PRO SER HIS ILE MET THR LYS
TCATATAATATCCAAAACAGAGAATGGTATATCCCTCTTCCCAGCCATATCATGACGAAA
AGTATATTATAGGTTTGTCTCTTACCATATAGGGAGAAGGGTCGGTAGTACTGCTTT
1090 1100 1110 1120 1130 1140

GLY ALA PKE LEU GLY GLY ALA ASP VAL LYS GLU CYS ILE GLU ALA PHE SER SER TYR ILE
GGGCATTCTAGGTGGGAGCAGATGTCAAGGAATGTATAGAAGCATTCAGCAGTTATATA
CCCGTAAAGATCCACCTCGTCTACAGTTCCTTACATATCTTCGTAAGTCGTCAATATAT
1150 1160 1170 1180 1190 1200

CYS PRO SER ASP PRO GLY PHE VAL LEU ASN HIS GLU KET GLU SER CYS LEU SER GLY ASN
TGCCCTTCTGATCCAGGATTGTACTAAACCATGAAATGGAGGAGCTGCTTATCAGGAAAC
ACGGGAAGACTAGGTCCTAAACATGATTGGTACTTTACCTTCGACGAATAGTCCTTTG
1210 1220 1230 1240 1250 1260

FIG.1D.

ILE SER GLN CYS PRO ARG THR [THR] VAL [THR] SER ASP ILE VAL PRO ARG TYR ALA PHE VAL ASN
ATATCCCAATGTCCAAGAACCACGGTCACACTCACATCAGAGATACACATTGTTCCAAGATATGCATTCGTC
TATAGGGTTACAGGTTCTTGGTGCCAGTGTGAGTCTGTAACAAGGTTCTATACGTAAGCAG
1270   1280   1290   1300   1310   1320

ASN GLY GLY VAL VAL ALA ASN CYS ILE THR THR THR CYS ASN GLY ILE [ASP]
AATGGAGGAGGTGGTTGCAAACTGTATAACAACCACCTGTACACATGCAACGGAATCGACAAT
TTACCTCCTCCACCAACGTTTGACATATTGTTGGTGGACATGTACGTTGCCTTAGCTGTTA
1330   1340   1350   1360   1370   1380

ARG ILE ASN GLN PRO PRO ASP GLN GLY VAL LYS ILE ILE THR HIS LYS GLU CYS ASN THR
AGAATCAACCAACCACCTGATCAAGGAGTAAAAATTATAACACATAAAGAATGTAATACA
TCTTAGTTGGTTGGTGGACTAGTTCCTCATTTTAATATTGTATTCTTACATTATGT
1390   1400   1410   1420   1430   1440

ILE GLY ILE ASN GLY MET LEU PHE ASN THR ASN LYS GLU GLY THR LEU ALA PHE TYR THR
ATAGGTATCAACGGAATGCTGTTCAATACAAATAAAGAAGGAACTCTTGCATTCTACACA
TATCCATAGTTGCCTTACGACAAGTTATGTTTATTTCCTTGAGAACGTAAGATGTGT
1450   1460   1470   1480   1490   1500

PRO ASN ASP ILE THR LEU ASN ASN SER VAL ALA LEU ASP PRO ILE SER ILE GLU GLU
CCAAATGATATAACAACTAAATAATTCTGTTGCACTTGATCCAATTGACATATCAATCGAG
GGTTTACTATATTGTTGATTTATTAAGACAACGTGAACTAGGTTAACTGTATAGTTAGCTC
1510   1520   1530   1540   1550   1560

LEU ASN LYS ALA LYS SER ASP LEU GLU GLU SER LYS GLU TRP ILE ARG ARG SER ASN GLN
CTTAACAAAGCCAAATCAGATCTAGAAGAATCAAAAGAATGGATAAGAAGGTCAAATCAA
GAATTGTTTCGGTTTAGTCTAGATCTTCTTAGTTTTCTTACCTATTCTTCCAGTTTAGTT
1570   1580   1590   1600   1610   1620

— TM —

LYS LEU ASP SER ILE GLY ASN TRP HIS GLN SER SER THR THR ILE ILE [ILE] LEU ILE
AAACTAGATTCTATTGGAAACTGGCATCAATCTAGCACTACAACTCATAATTATTTTAATA
TTTGATCTAAGATAACCTTTGACCGTAGTCGTTAGATCGTTGAGTGTTATTAATAAAATTAT
1630   1640   1650   1660   1670   1680

FIG.1E

MET ILE ILE ILE LEU PHE ILE ILE ASN VAL THR ILE ILE [THR] ILE ALA [ILE] LYS TYR TYR
ATGATCATTATTATTGTTTATAATTAATTAATGTAACGATAATTACAATTGCAATTAAGTATTAC
TACTAGTAATAATAACAAATATTAATTACATTGCTATTAATGTTAACGTTAATTCATAATG
    1690   1700   1710   1720   1730   1740

ARG ILE GLN LYS ARG ASN ARG VAL ASP ASP GLN ASN ASP LYS PRO TYR VAL LEU THR ASN LYS
AGAATTCAAAAGAGAAATCGAGTGGATCAAAATGACAAGCCATATGTACTAACAACAAA
TCTTAAGTTTTCTCTTTAGCTCACCTAGTTTTACTGTTCGGTATACATGATTGTTTGTTT
    1750   1760   1770   1780   1790   1800

TGACATATCTATAGATCATTAGATATTAAAATTATAAAAAACTT
ACTGTATAGATATCTAGTAATCTATAATTTAATATTTTTGAA
    1810   1820   1830   1840

NUCLEOTIDE SEQUENCE OF THE PIV-3 F GENE. THE cDNA SEQUENCE
IS SHOWN IN THE PLUS (mRNA) STRAND SENSE IN THE 5' TO 3'
DIRECTION. THE SIGNAL PEPTIDE (SP) AND THE TRANSMEMBRANE (TM)
ANCHOR DOMAIN ARE UNDERLINED. THE PREDICTED F2-FI CLEAVAGE SITE IS
INDICATED BY THE ARROW (↓). AMINO ACIDS DIFFERING FROM THE
PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY THE PIV-3 F
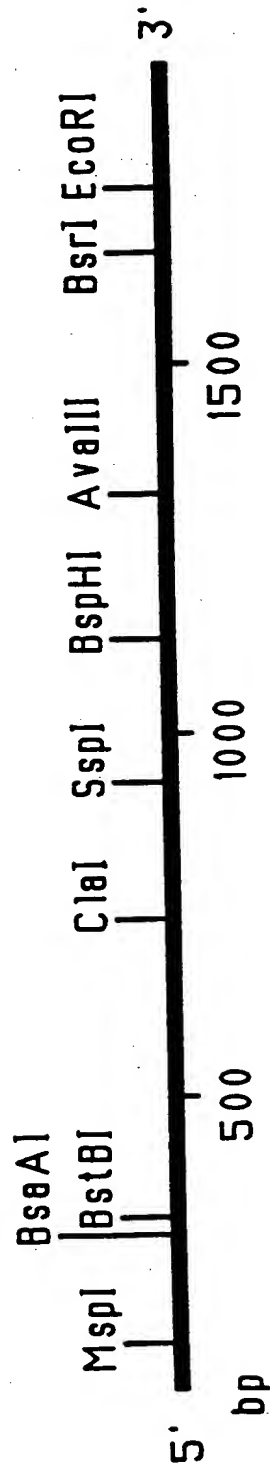GENE ARE BOXED.

RESTRICTION MAP OF THE PIV-3 F GENE

FIG.2.

FIG.3A.

NUCLEOTIDE SEQUENCE OF THE PIV-3 HN GENE.

```
                MET GLU TYR TRP LYS HIS THR ASN HIS GLY LYS ASP ALA GLY
5' AGACAAATTCCAAATTCGAGATGGAATACTGGAAGCATACCAATCACGGAAAAGGATGCTGG
   TCTGTTTAAGGTTTAAGCTCTACCTTATGACCTTCGTATGGTTAGTGCCTTCCTACGACC
            10          20          30          40          50          60
                                                            ───TM──→

   ASN GLU LEU GLU THR SER MET ALA THR [ASN] GLY ASN LYS [LEU] THR ASN LYS ILE THR TYR
   CAATGAGCTGGAGACGTCCATGGCTACTAATGGCAACAAGCTCACCAATAAGATAACATA
   GTTACTCGACCTCTGCAGGTACCGATGATTACCGTTGTTCGAGTGGTTATTCTATTGTAT
            70          80          90         100         110         120

   ILE LEU TRP THR ILE ILE LEU VAL LEU LEU SER ILE VAL PHE ILE ILE VAL LEU ILE ASN
   TATTATTGGACAATAATCCTGGTGTTATTATCAATAGTCTTCATCATAGTGCTAATTAA
   ATATAATACCTGTTATTAGGACCACAATAATAGTTATCAGAAGTAGTATCACGATTAATT
           130         140         150         160         170         180

   SER ILE LYS SER GLU LYS ALA HIS GLU SER LEU LEU GLN ASP [ILE] ASN ASN GLU PHE MET
   TTCCATCAAAAGTGAAAAGGCTCATGAATCATTGCTGCAAGACATAAATAATGAGTTAT
   AAGGTAGTTTCACTTTTCCGAGTACTTAGTAACGACGTTCTGTATTTATTACTCAAATA
           190         200         210         220         230         240

   GLU [ILE] THR GLU LYS ILE GLN MET ALA SER ASP ASN [THR] ASN ASP LEU ILE GLN SER GLY
   GGAATTACAGAAAAGATCCAAATGGCATCGGATAATACCAATGATCTAATACAGTCAGG
   CCTTTAATGTCTTTTTCTAGGTTTACCGTAGCCTATTATGGTTACTAGATTATGTCAGTCC
           250         260         270         280         290         300

   VAL ASN THR ARG LEU LEU THR ILE GLN SER HIS VAL GLN ASN TYR ILE PRO ILE SER LEU
   AGTGAATACAAGGCTTCTTACAATTCAGAGTCATGTCCAGAATTATATACCAATATCACT
   TCACTTATGTTCCGAAGAATGTTAAGTCTCAGTACAGGTCTTAATATATGGTTATAGTGA
           310         320         330         340         350         360
```

THR GLN GLN MET SER ASP LEU ARG LYS PHE ILE SER GLU ILE THR ARG ASN ASP ASN
GACACACAGATGTCTACAGTCTTAGGAAATTCATTAGTGAATTACAATTAGAAATGATAA
CTGTGTGTCTACAGATGTCAGAGTCTTAGGAAATTCATTAGTTAATCTTTACTATT
370   380   390   400   410   420

[GLN] GLU VAL [LEU] PRO GLN ARG ILE THR HIS ASP [VAL] GLY ILE LYS PRO LEU ASN PRO ASP
TCAAGAAGTGCTGCCACAAAGAATAACACATGATGTGGGTATAAAACCTTAAATCCAGA
AGTTCTTCACGACGGTGTTTCTTATTGTGTACTACACCCATATTTTGGAAATTTAGGTCT
430   440   450   460   470   480

ASP PHE TRP ARG CYS THR SER GLY LEU PRO SER LEU MET LYS THR PRO LYS ILE ARG LEU
TGATTTTGGAGATGCACGTCTGGTCTTCCATCTTTAATGAAAACTCCAAAATAAGGTT
ACTAAAAACCTCTACGTGCAGACCAGAGGTAGAAATTACTTTGAGGTTTTATTCCAA
490   500   510   520   530   540

MET PRO GLY PRO GLY LEU LEU ALA MET PRO THR THR VAL ASP GLY CYS [ILE] ARG THR PRO
AATGCCAGGGCCCGGGCCCCTAATAATGCTATGCCAACGACTGTTGATGGCTGTATCAGAACTCC
TTACGGTCCCGGGCCCGGGATTATTAGCTATACGGTTGCTGACAACTACCGACATAGTCTTGAGG
550   560   570   580   590   600

SER LEU VAL ILE ASN ASP LEU ILE TYR THR ALA TYR THR SER ASN LEU ILE THR ARG GLY CYS
GTCCTTAGTTATAAATGATCTGATTATGCTTATACCTCAATCCAATTACTCGAGGTTG
CAGGAATCAATATTTACTAGACTAAATACGAATATGGAGTTTAGGTTAATGAGCTCCAAC
610   620   630   640   650   660

GLN ASP ILE GLY LYS SER TYR GLN VAL LEU GLN ILE GLY ILE THR VAL ASN SER ASP
TCAGGATATAGGAAAATCATATCAAGTCTTACAGATAGGGATAATAACTGTAAACTCAGA
AGTCCTATATCCTTTTTAGTATAGTTCAGAATGTCTATCCCTATTATTGACATTTGAGTCT
670   680   690   700   710   720

LEU VAL PRO ASP LEU ASN PRO ARG ILE SER HIS THR PHE ASN ILE ASN ASP ASN ARG LYS
CTTGGTACCTGACTTAAATCCCAGGATCTTCATACTTTTAAACATAAAATGACAATAGGAA
GAACCATGGACTGAATTTAGGGGTCCTAGAGAGTATGAAAATTGTTATTTACTGTATCCTT
730   740   750   760   770   780

FIG.3B.

SRE CYS SER LEU ALA LEU LEU ASN THR ASP VAL TYR GLN LEU CYS SER THR PRO LYS VAL
GTCATGTTCTCTAGCACTCCTAAATACAGATGTATACATATAGTTCAACTGTGTCAACTCCCAAAGT
CAGTACAAGAGATCGTGAGGATTTATGTCTACATATAGTTGACAAGTTGAGGGTTCA
790　　　　800　　　　810　　　　820　　　　830　　　　840

ASP GLU ARG SER ASP TYR ALA SER SER GLY ILE GLU ASP ILE VAL LEU ASP ILE VAL ASN
TGATGAAAGATCAGATTATGCATCAGGCATAGAAGATATTGTACTTGATATTGTCAA
ACTACTTTCTAGTCTAATACGTAGTCCGTATCTTCTATAACATGAACTATAACAGTT
850　　　　860　　　　870　　　　880　　　　890　　　　900

[TYR] ASP GLY SER ILE SER THR THR ARG PHE LYS ASN ASN ILE SER PHE ASP GLN PRO
TTATGATGGCTCAATCTCAACAACAAGATTTAAGAATAATAACATAAGCTTTGATCAACC
AATACTACCGAGTTAGAGTTGTTGTTCTAAATTCTTATTATTGTTCCGTTTATTATAA
910　　　　920　　　　930　　　　940　　　　950　　　　960

TYR ALA ALA LEU TYR PRO SER VAL GLY PRO GLY ILE TYR TYR LYS ILE ILE PHE
TATGCTGCACTATACCCATCTGTTGGACCAGGGATATACTACAAGGCAAAATAATATT
AATACGACGTGATATGGGTAGACAACCTGGTCCCTATATGATGTTCCGTTTTATTATAA
970　　　　980　　　　990　　　　1000　　　　1010　　　　1020

LEU GLY TYR GLY GLY LEU GLU HIS PRO ILE ASN GLU ASN [VAL] ILE CYS ASN THR THR GLY
TCTCGGGTATGGAGGTCTTGAACATCCAATAAATGAGAATGTAATCTGCAACAACTGG
AGAGCCCATACCTCCAGAACTTGTAGGTTATTTACTCTTACATTAGACGTTGTTGACC
1030　　　　1040　　　　1050　　　　1060　　　　1070　　　　1080

CYS PRO GLY LYS THR GLN ARG ASP CYS ASN GLN ALA SER HIS SER PRO TRP PHE SER ASP
GTGTCCGGGAAAACACAGAGAGACTGCAATCAGGCATCTCATAGTCCATGGTTTTCAGA
CACAGGGCCCTTTTGTGTCTCTCTGACGTTAGTCCGTAGAGTATCAGGTACCAAAGTCT
1090　　　　1100　　　　1110　　　　1120　　　　1130　　　　1140

ARG ARG MET VAL ASN SER ILE ILE VAL VAL ASP LYS GLY LEU ASN SER ILE PRO LYS LEU
TAGGAGGATGGTCAACTCTATCATTGTTGACAAAGGCTTAAACTCAATTCCAAATT
ATCCTCCTACCAGTTGAGATAGTAACAACAACTGTTTCCGAATTTGAGTTAAGGTTTTAA
1150　　　　1160　　　　1170　　　　1180　　　　1190　　　　1200

FIG.3C.

```
LYS VAL TRP THR ILE SER MET ARG GLN ASN TYR TRP GLY SER GLU GLY ARG LEU LEU LEU
GAAGGTATGGACGATATCTATGAGACAGAATTACTGGGGGTCAGAAGGAAGGTTACTTCT
CTTCCATACCTGCTATAGATACTCTGTCTTAATGACCCCCAGTCTTCCTTCCAATGAAGA
      1210        1220        1230        1240        1250        1260

LEU GLY ASN LYS ILE TYR ILE TYR THR ARG SER THR SER LYS TRP HIS SER LYS LEU GLN LEU
ACTAGGTAACAAGATCTATATATACAAGATCCACAGTTGGCATAGACAAGTTACAATT
TGATCCATTGTTCTAGATATATATATTTCTAGGTGTTCAACGTATCGTTCAATGTTAA
      1270        1280        1290        1300        1310        1320

GLY ILE ASP ILE THR ASP TYR SER ASP ILE ARG ILE LYS TRP THR TRP HIS ASN VAL
AGGAATAATTGATATTACTGATTACAGTGATATAAGGATATATATTCCTATTTCACTTACA
TCCTTATTAACTATAATGACTAATGTCACTATATTCCTATATTTACCTGTACCGTATTACA
      1330        1340        1350        1360        1370        1380

LEU SER ARG PRO GLY ASN ASN GLU CYS TRP GLY HIS SER CYS PRO ASP GLY CYS ILE
GCTATCAAGACCAGGAAACAATGAATGTCCATGGGACATTCATGTCCAGATGGATGTAT
CGATAGTTCTGGTCCTTTGTTACTTACAGGTACCCTGTAAGTACAGGTCTACCTACATA
      1390        1400        1410        1420        1430        1440

THR GLY VAL TYR THR ASP ALA TYR PRO LEU ASN PRO THR GLY SER ILE VAL SER SER VAL
AACAGGAGTATATACTGATGCCATATCCAATCCACCACAGGGAGCATTGTGTCATCTGT
TTGTCCTCATATATGACTACGGTATAGGTTAGGTGGTGTCCCTCGTAACACAGTAGACA
      1450        1460        1470        1480        1490        1500

ILE LEU ASP SER GLN LYS SER ARG VAL ASN PRO VAL ILE THR TYR TYR SER THR [ALA] THR GLU
CATATTAGATTCACAAAAATCGAGAGTGAACCCAGTCATAACTTACTTGAATGAGTTGGCT
GTATAATCTAAGTGTTTTTAGCTCTCACTTGGGTCAGTATTGAATGAACTTACTCAACCGA
      1510        1520        1530        1540        1550        1560

ARG VAL ASN GLU LEU ALA ILE [ARG] ASN ARG THR LEU SER ALA GLY TYR THR THR THR SER
AAGAGTAAACGAGCTGGCCATCCGAAACAACAGAACACTCCAGCTGGATATACAACAAG
TTCTCATTTGCTCGACCGGTAGGCTTTGTTGTCTTGTGAGAGTCGACCTATATGTTGTTC
      1570        1580        1590        1600        1610        1620
```

FIG.3D.

CYS ILE THR HIS TYR ASN LYS GLY TYR CYS PHE HIS ILE VAL GLU ILE ASN [GLN] LYS SER
CTGCATCCACACTATAACAAAGGATATTGTTTTCATATAGTAGAAATAAATCAGAAAAG
GACGTAGTGTGTGATATTGTTTCCTATAACAAAAGTATATCATCTTTATTTAGTCTTTC
1630   1640   1650   1660   1670   1680

LEU [ASN] THR [LEU] GLN PRO MET LEU PHE LYS THR GLU [VAL] PRO LYS SER CYS SER ***
CTTAAACACTTCAACCCATGTTGTTCAAGAGACAGAGGTTCCAAAAGCTGCAGTTAATC
GAATTTGTGTGAAGTTGGGTACAACAAGTTCTGTCTCTCCAAGGTTTTTCGACGTCAATTAG
1690   1700   1710   1720   1730   1740

ATAATTAACCGCAATATGCATTAACCTATCTATAATACAAGTATATATGATAAGTAATCAGC
TATTAATTGGCGTTATACGTAATTGGATAGATATTATGTTCATTACTATTCATTAGTCG
1750   1760   1770   1780   1790   1800

AATCAGACAATAGACAAAAGGGAAATATAAAAA
TTAGTCTGTTATCTGTTTTCCCTTTATATTTT
1810   1820   1830

NUCLEOTIDE SEQUENCE OF THE PIV-3 HN GENE. THE cDNA SEQUENCE
IS SHOWN IN THE PLUS (mRNA) STRAND SENSE IN THE 5' TO 3'
DIRECTION. THE TRANSMEMBRANE (TM) ANCHOR DOMAIN IS UNDERLINED. AMINO ACIDS
DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY THE PIV-3
HN GENE ARE BOXED.

FIG.3E.

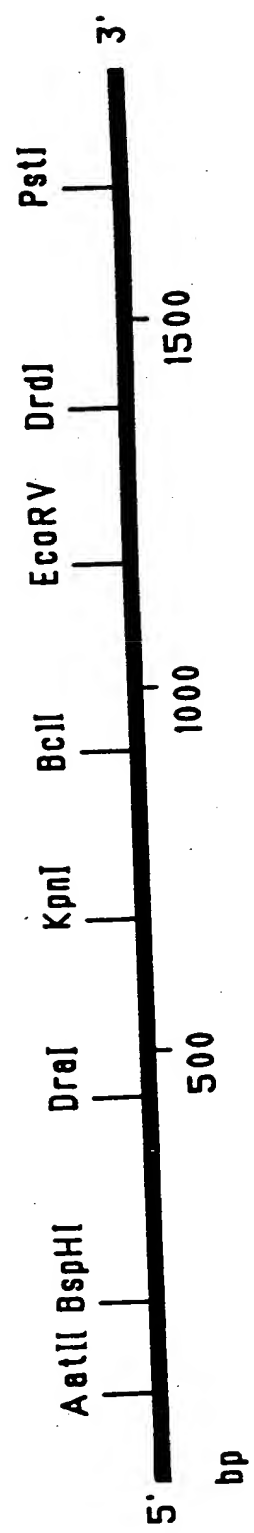RESTRICTION MAP OF THE PIV-3 HN GENE

FIG.4.

FIG.5A.

NUCLEOTIDE SEQUENCE OF THE RSV F GENE.

—— SP ——→

```
5'MET GLU LEU [PRO] ILE LEU LYS ALA ASN ALA ILE THR THR ILE LEU ALA [ALA] VAL THR PHE
  ATGGAGTTGCCAATCCTCAAGCAAATGCAAATTACCACAATCCTCGCTGCCAGTCACATTT
  TACCTCAACGGTTAGGAGTTCGTTTACGTTAATGGTGTTAGGAGCGACGTCAGTGTAAA
               10            20            30            40            50            60

CYS PHE ALA [SER] SER GLN ASN ILE THR GLU GLU PHE TYR GLN SER THR CYS SER ALA VAL
  TGCTTTGCTTCTAGTCAAAACATCACTGAAGAATTTATCAATCAACATGCAGTCAGTT
  ACGAAACGAAGATCAGTTTGTAGTGACTTCTTAAAATAGTTAGTTGTACGTCACGTCAA
               70            80            90            100           110           120

SER LYS GLY TYR LEU SER ALA LEU ARG THR GLY TRP TYR THR SER VAL ILE THR ILE GLU
  AGCAAAGGCTATCTTAGTGCTCTAAGAACTGGTTGGTATACTAGTGTTATAACTATAGAA
  TCGTTTCCGATAGAATCACGAGATTCTTGACCAACCATATGATCACAATATTGATATCTT
               130           140           150           160           170           180

LEU SER ASN ILE LYS GLU ASN LYS CYS ASN GLY THR ASP ALA LYS VAL LYS LEU [MET] LYS
  TTAAGTAATATCAAGGAAAATAAGTGTAATGGAACAGATGCTAAGGTAAAAATTGATGAAA
  AATTCATTATAGTTCCTTTATTCACATTACCTTGTCTACGATTCCATTTTAACTACTTT
               190           200           210           220           230           240

GLN GLU LEU ASP LYS TYR LYS ASN ALA VAL THR GLU LEU GLN LEU LEU MET GLN SER THR
  CAAGAATTAGATAAATATAAAAATGCTGTAACAGAATTGCAGTTGCTCATGCAAAGCACA
  GTTCTTAATCTATTTATATTTTACGACATTGTCTTAACGTCAACGAGTACGTTTCGTGT
               250           260           270           280           290           300

PRO [ALA] ALA ASN ASN ARG ALA ARG ARG GLU LEU PRO ARG PHE MET ASN TYR THR LEU ASN
  CCAGCAGCAAACAATCGAGCCAGAAGAGAACTACCAAGGTTATGAATTATACACTCAAC
  GGTCGTCGTTTGTTAGCTCGGTCTTCTCTTGATGGTTCCAAATACTTATAATGTGAGTTG
               310           320           330           340           350           360
```

```
                                                         F2-F1 CLEAVAGE SITE
ASN [THR] LYS LYS THR ASN VAL THR LEU SER LYS LYS ARG ARG↓PHE LEU GLY PHE
AATACCAAAAAAACCAATGTAACATTAAGCAAGAAAAAGGAAAGAAGAAGATTCTTGGTTTT
TTATGGTTTTTTTGGTTACATTGTAATTCGTTCTTTTCCTTTCTTCTTCTAAGAACCAAAA
        370        380        390        400        410        420

LEU LEU GLY VAL GLY SER ALA ILE ALA SER GLY [ILE] ALA VAL SER LYS VAL LEU HIS LEU
TTGTTAGGTGGTTGGATCTGCAATCGCCAGTGGCATTGCTGTATCTAAGGTCCTGCACTTA
AACAATCCACCAACCTAGACGTTAGCGGTCACCGTAACGACATAGATTCCAGGACGTGAAT
        430        440        450        460        470        480

GLU GLY VAL ASN LYS ILE LYS SER ALA LEU LEU SER THR ASN LYS ALA VAL VAL SER
GAAGGAGAAGTGAACAAGATCAAAAGTGCTCTACTATCCACAAACAAGGCCGTAGTCAGT
CTTCCTCTTCACTTGTTCTAGTTTTCACGAGATGATAGGTGTTTGTTCCGGCATCAGTCA
        490        500        510        520        530        540

LEU SER ASN GLY VAL SER VAL LEU THR SER LYS VAL LEU ASP LEU LYS ASN TYR ILE ASP
TTATCAAATGGAGTTAGTGTCTTAACCAGCAAAGTGTTAGACCTCAAAAACTATATAGAT
AATAGTTTACCTCAATCACAGAATTGGTCGTTTCACAATCTGGAGTTTGATATATCTA
        550        560        570        580        590        600

LYS GLN LEU LEU PRO ILE VAL ASN LYS ARG SER CYS [ARG] ILE SER ASN ILE GLU THR VAL
AAACAATTGTTACCTATTGTGAATAAGCGGAAGCTGCAGAATATCAAATATAGAAACTGTG
TTTGTTAACAATGGATAACACTTATTCGCCTTCGACGTCTTATAGTTTATATCTTTGACAC
        610        620        630        640        650        660

ILE GLU PHE GLN HIS LYS ASN ASN ARG LEU LEU GLU ILE THR ARG GLU PHE SER VAL ASN
ATAGAGTTCCAACAACAAGAACAACAGACTACTAGAGATTACCAGGAATTTAGTGTTAAT
TATCTCAAGGTTGTTGTTCTTGTTGTCTGATGATCTCTAATGGTCCCTTAAATCACAATTA
        670        680        690        700        710        720

ALA GLY VAL THR THR PRO VAL SER THR TYR MET LEU THR ASN SER GLU LEU LEU SER LEU
GCAGGTGTAACTACACCTGTAAGCACTTACATTACTAATAGTGAATTATTGTCATTA
CGTCCACATTGATGTGGACATTCGTGAATGTAATGATTATCACTTAATAACAGTAAT
        730        740        750        760        770        780
```

FIG.5B.

ILE ASN ASP MET PRO ILE THR ASN ASP GLN LYS LYS LEU MET SER ASN ASN VAL GLN ILE
ATCAATGATATGCCTATAACAAATGATCAGAAAAAGTTAATGTCCAACAATGTTCAAATA
TAGTTACTATACGGATATTGTTTACTAGTCTTTTTCAATTACAGGTTGTTACAAGTTTAT
790   800   810   820   830   840

VAL ARG GLN GLN SER TYR SER ILE MET SER ILE ILE LYS GLU GLU VAL LEU ALA TYR VAL
GTTAGACAGCAAAGTTACTCTATCATGTCCATAATAAAAGAGGAAGTCTTAGCATAGTA
CAATCTGTCGTTTCAATGAGATAGTACAGGTATTATTTTTCCTTCAGAATCGTATACAT
850   860   870   880   890   900

VAL GLN LEU PRO LEU TYR GLY VAL ILE ASP THR PRO CYS TRP LYS LEU HIS THR SER PRO
GTACAATTACCACTATATGGTGTGATAGATACACCCTGTTGGAAATTACACATCCCT
CATGTTAATGGTGATATACCACACTATCTATGTGGGACAACCTTTAATGTGTAGGGGA
910   920   930   940   950   960

LEU CYS THR THR ASN THR LYS GLU GLY SER ASN ILE CYS LEU THR ARG THR ASP ARG GLY
CTATGTACAACCAACACAAAGAAGGGTCAAACATCTGTTAACAAGAACTGACAGAGGA
GATACATGTTGGTTGTGTTTCTTCCCAGTTTGTAGACAATTGTTCTTGACTGTCTCCT
970   980   990   1000   1010   1020

TRP TYR CYS ASP ASN ALA GLY SER VAL SER PHE PHE PRO GLN ALA GLU THR CYS LYS VAL
TGGTACTGTGACAATGCAGGATCAGGATCAGTATCTTCTCCACAAGCTGAAACATGTAAAGTT
ACCATGACACTGTTACGTCCTAGTCCTAGTCATAGAAGAAGAGAAGGGTGTTCGACTTTGTACATTCAA
1030   1040   1050   1060   1070   1080

GLN SER ASN ARG VAL PHE CYS ASP THR MET ASN SER LEU THR LEU PRO SER GLU [VAL] ASN
CAATCGAATCGAGTATTTGTGACAATGAACAGTTAACATTACCAAGTGAAGTAAAT
GTTAGCTTAGCTCATAAAACACTGTTACTTGTCAAATTGTAATGGTTCACTTCATTTA
1090   1100   1110   1120   1130   1140

LEU CYS ASN VAL ASP ILE PHE ASN PRO LYS TYR ASP CYS LYS ILE MET THR SER LYS THR
CTCTGCAATGTTGACATATTCAATCCCAAATATGATTGTAAAATTATGACTTCAAAAACA
GAGACGTTACAACTGTATAAGTTAGGGTTTATACTAACATTTTAATACTGAAGTTTTGT
1150   1160   1170   1180   1190   1200

FIG.5C.

ASP VAL SER SER VAL ILE THR SER LEU GLY ALA ILE VAL SER CYS TYR GLY LYS THR
GATGTAAGCAGCTCCGTTATCACATCTCTAGGAGCCATTGTGTCATGCTATGGCAAAACT
CTACATTCGTCGGAGGCAATAGTGTAGAGATCCTCGGTAACACAGTACGATACCGTTTTGA
        1210   1220   1230   1240   1250   1260

LYS CYS THR ALA SER ASN LYS ASN ARG GLY ILE ILE LYS THR PHE SER ASN GLY CYS ASP
AATGTACAGCATCCAATAAAAAATCGTGGAATCATAAAGACATTTCTAACGGGTGTGAT
TTACATGTCGTAGGTTATTTTTTAGCACCTTAGTATTTCTGTAAAAGATTGCCCACACTA
        1270   1280   1290   1300   1310   1320

TYR VAL SER ASN LYS GLY [VAL] ASP THR VAL SER VAL GLY ASN THR LEU TYR TYR VAL ASN
TATGTATCAAATAAAGGGGTGGACACTGTGTCTGTAGGTAACACATTATTATATGTAAAT
ATACATAGTTTATTTCCCACCTGTGACACAGACATCCATTGTGTAATAATACATTTA
        1330   1340   1350   1360   1370   1380

LYS GLN GLU GLY LYS SER LEU TYR VAL LYS GLY GLU PRO ILE ILE ASN PHE TYR ASP PRO
AAGCAAGAGGGCAAAGTCTCTATGTAAAAGGTGAACAATAAATTCTATGACCCA
TTCGTTCTTCCGTTTTCAGAGATACATTTCCACTTGGTTATTTAAAGATACTGGGT
        1390   1400   1410   1420   1430   1440

LEU VAL PHE PRO SER ASP GLU PHE ASP ALA SER ILE SER GLN VAL ASN GLU LYS ILE ASN
TTAGTATTCCCTCTGATGAATTGCATCAATATCTCAAGTCAACGAGAAGATTAAC
AATCATAAGGGGGAGACTACTTAAAACTACGTAGTTATAGAGTTCAGTTGCTCTTCTAATTG
        1450   1460   1470   1480   1490   1500

GLN SER LEU ALA PHE ILE ARG LYS SER ASP GLU LEU LEU HIS ASN VAL ASN ALA GLY LYS
CAGAGTTAGCATTTATTCGTAAATCCGATAAATTACATAATGTAAATGCTGGTAAA
GTCTCAAATCGTAAATAAGCATTTAGGCTACTTAATAATGTATTACGACCATTT
        1510   1520   1530   1540   1550   1560

TM
—————————→

SER THR THR ASN ILE THR ILE MET ILE ILE GLU ILE ILE VAL ILE LEU LEU LEU SER
TCAACCAAATATCATGATAACTACTATTATTATTAGAGATTAATATTGTTATCA
AGTTGGTGTTTATAGTACTTATTGATGATTAATATCTTAATTCTTAATAACAATAGT
        1570   1580   1590   1600   1610   1620

FIG.5D.

LEU ILE ALA VAL GLY LEU LEU LEU TYR CYS LYS ALA ARG SER THR PRO VAL THR LEU SER
TTAATTGCTGTTGGACTGCTCCTATACTGTAAGGCCAGAAGCACACGAGTCACACTAAGC
AATTAACGACAACCTGACGAGGATATGACATTCCGGTCTTCGTGTGTGGTCAGTGTGATTCG
1630   1640   1650   1660   1670   1680

LYS ASP GLN LEU SER GLY ILE ASN ASN ILE ALA PHE SER ASN
AAGGATCAACTGAGTGGTATAAATAATAATATTGCATTTAGTAACTGAATAAAAATAGCACCT
TTCCTAGTTGACTCACCATATTTATTATTATAACGTAAATCATTGACTTATTTTATCGTGGA
1690   1700   1710   1720   1730   1740

AATCATGTTCTTACAATGGTTTACTATCTGCTCATAGACAACCCATCTATCATTGGATTT
TTAGTACAAGAATGTTACCAAATGATAGAGCGTAGTATCGTGTGGGTAGATAGTAACCTAAA
1750   1760   1770   1780   1790   1800

TCTTAAAATCTGAACTTCATCGAAACTCTTATCTATAAACCATCTCACTTACACTATTTA
AGAATTTTAGACTTGAAGTAGCTTTGAGAATAGATATTTGGTAGAGTGAATGTGATAAAAT
1810   1820   1830   1840   1850   1860

AGTAGATTCCTAGTTTATAGTTATAT 3'
TCATCTAAGGATCAAATATCAAATATA
1870   1880

NUCLEOTIDE SEQUENCE OF THE RSV F GENE. THE cDNA SEQUENCE IS SHOWN IN THE PLUS (mRNA)
STRAND SENSE IN THE 5' TO 3' DIRECTION. THE SIGNAL PEPTIDE (SP) AND THE TRANSMEMBRANE (TM)
ANCHOR DOMAIN ARE UNDERLINED. THE PREDICTED F2-F1 CLEAVAGE SITE IS INDICATED BY THE ARROW
(↓). AMINO ACIDS DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF THE PROTEIN ENCODED BY
THE RSV F GENE ARE BOXED.

FIG.5E.

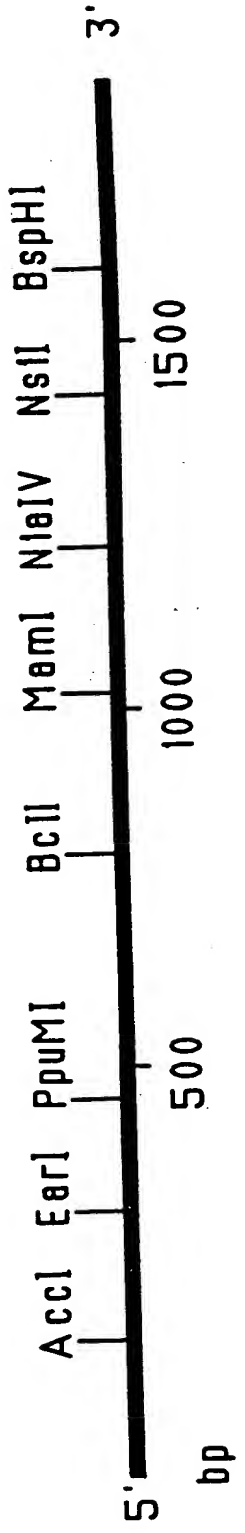RESTRICTION MAP OF THE RSV F GENE

FIG.6.

FIG.7A. NUCLEOTIDE SEQUENCE OF THE RSV G GENE

```
        MET   SER   LYS   ASN   LYS   ASP   GLN   ARG
T G C A A A C A T G T C C A A A A A C A A G G A C C A A C G
A C G T T T G T A C A G G T T T T T G T T C C T G G T T G C
            10                20                30
```

```
        THR   ALA   LYS   THR   LEU   GLU  [LYS]  THR   TRP   ASP
C A C C G C T A A G A C A C T A G A A A A G A C C T G G G A
G T G G C G A T T C T G T G A T C T T T T C T G G A C C C T
            40                50                60
```

```
        THR   LEU   ASN   HIS   LEU   LEU   PHE   ILE   SER   SER
C A C T C T C A A T C A T T T A T T A T T C A T A T C A T C
G T G A G A G T T A G T A A A T A A T A A G T A T A G T A G
            70                80                90
                                              ←
```

```
       [GLY]  LEU   TYR   LYS   LEU   ASN   LEU   LYS   SER   VAL
G G G C T T A T A T A A G T T A A A T C T T A A A T C T G T
C C C G A A T A T A T T C A A T T T A G A A T T T A G A C A
            100               110               120
──────────────────────── TM ────────────────────────
```

```
        ALA   GLN   ILE   THR   LEU   SER   ILE   LEU   ALA   MET
A G C A C A A A T C A C A T T A T C C A T T C T G G C A A T
T C G T G T T T A G T G T A A T A G G T A A G A C C G T T A
            130               140               150
```

```
        ILE   ILE   SER   THR   SER   LEU   ILE   ILE  [THR]  ALA
G A T A A T C T C A A C T T C A C T T A T A A T T A C A G C
C T A T T A G A G T T G A A G T G A A T A T T A A T G T C G
            160               170               180
                                                  ─────→
```

```
        ILE   ILE   PHE   ILE   ALA   SER   ALA   ASN   HIS   LYS
C A T C A T A T T C A T A G C C T C G G C A A A C C A C A A
G T A G T A T A A G T A T C G G A G C C G T T T G G T G T T
            190               200               210
```

```
        VAL   THR  [LEU]  THR   THR   ALA   ILE   ILE   GLN   ASP
A G T C A C A C T A A C A A C T G C A A T C A T A C A A G A
T C A G T G T G A T T G T T G A C G T T A G T A T G T T C T
            220               230               240
```

```
        ALA   THR   SER   GLN   ILE   LYS   ASN   THR   THR   PRO
T G C A A C A A G C C A G A T C A A G A A C A C A A C C C C
A C G T T G T T C G G T C T A G T T C T T G T G T T G G G G
            250               260               270
```

```
        THR   TYR   LEU   THR   GLN  [ASP]  PRO   GLN   LEU   GLY
A A C A T A C C T C A C T C A G G A T C C T C A G C T T G G
T T G T A T G G A G T G A G T C C T A G G A G T C G A A C C
            280               290               300
```

FIG.7B.

```
     ILE   SER  [PHE]  SER   ASN  [LEU]  SER   GLU   ILE   THR
   A A T C A G C T T C T C C A A T C T G T C T G A A A T T A C
   T T A G T C G A A G A G G T T A G A C A G A C T T T A A T G
               310               320               330

         SER   GLN  [THR]  THR   THR   ILE   LEU   ALA   SER   THR
   A T C A C A A A C C A C C A C C A T A C T A G C T T C A A C
   T A G T G T T T G G T G G T G G T A T G A T C G A A G T T G
               340               350               360

         THR   PRO   GLY   VAL   LYS   SER  [ASN]  LEU   GLN  [PRO]
   A A C A C C A G G A G T C A A G T C A A A C C T G C A A C C
   T T G T G G T C C T C A G T T C A G T T T G G A C G T T G G
               370               380               390

         THR   THR   VAL   LYS   THR   LYS   ASN   THR   THR   THR
   C A C A A C A G T C A A G A C T A A A A A C A C A A C A A C
   G T G T T G T C A G T T C T G A T T T T T G T G T T G T T G
               400               410               420

         THR   GLN   THR   GLN   PRO   SER   LYS   PRO   THR   THR
   A A C C C A A A C A C A A C C C A G C A A G C C C A C T A C
   T T G G G T T T G T G T T G G G T C G T T C G G G T G A T G
               430               440               450

         LYS   GLN   ARG   GLN   ASN   LYS   PRO   PRO  [ASN]  LYS
   A A A A C A A C G C C A A A A C A A A C C A C C A A A C A A
   T T T T G T T G C G G T T T T G T T T G G T G G T T T G T T
               460               470               480

         PRO   ASN   ASN   ASP   PHE   HIS   PHE   GLU   VAL   PHE
   A C C C A A T A A T G A T T T T C A C T T C G A A G T G T T
   T G G G T T A T T A C T A A A A G T G A A G C T T C A C A A
               490               500               510

         ASN   PHE   VAL   PRO   CYS   SER   ILE   CYS   SER   ASN
   T A A C T T T G T A C C C T G C A G C A T A T G C A G C A A
   A T T G A A A C A T G G G A C G T C G T A T A C G T C G T T
               520               530               540

         ASN   PRO   THR   CYS   TRP   ALA   ILE   CYS   LYS   ARG
   C A A T C C A A C C T G C T G G G C T A T C T G C A A A A G
   G T T A G G T T G G A C G A C C C G A T A G A C G T T T T C
               550               560               570

         ILE   PRO   ASN   LYS   LYS   PRO   GLY   LYS   LYS   THR
   A A T A C C A A A C A A A A A A C C A G G A A A G A A A A C
   T T A T G G T T T G T T T T T T G G T C C T T T C T T T T G
               580               590               600
```

FIG.7C.

```
      THR   THR   LYS   PRO   THR   LYS   LYS   PRO   THR   PHE
    C A C C A C C A A G C C T A C A A A A A A A A C C A A C C T T
    G T G G T G G T T C G G A T G T T T T T T T G G T T G G A A
                    610                 620                 630

      LYS   THR   THR   LYS   LYS   ASP  [LEU]  LYS   PRO   GLN
    C A A G A C A A C C A A A A A A G A T C T C A A A C C T C A
    G T T C T G T T G G T T T T T T C T A G A G T T T G G A G T
                    640                 650                 660

      THR   THR   LYS  [PRO]  LYS   GLU   VAL   PRO   THR   THR
    A A C C A C T A A A C C A A A G G A A G T A C C C A C C A C
    T T G G T G A T T T G G T T T C C T T C A T G G G T G G T G
                    670                 680                 690

      LYS   PRO   THR   GLU   GLU   PRO   THR   ILE   ASN   THR
    C A A G C C C A C A G A A G A G C C A A C C A T C A A C A C
    G T T C G G G T G T C T T C T C G G T T G G T A G T T G T G
                    700                 710                 720

      THR   LYS   THR   ASN   ILE  [THR]  THR   THR   LEU   LEU
    C A C C A A A A C A A A C A T C A C A A C T A C A C T G C T
    G T G G T T T T G T T T G T A G T G T T G A T G T G A C G A
                    730                 740                 750

      THR  [ASN]  ASN   THR   THR   GLY   ASN   PRO  [LYS]  LEU
    C A C C A A C A A C A C C A C A G G A A A T C C A A A A C T
    G T G G T T G T T G T G G T G T C C T T T A G G T T T T G A
                    760                 770                 780

      THR   SER   GLN   MET   GLU   THR   PHE   HIS   SER   THR
    C A C A A G T C A A A T G G A A A C C T T C C A C T C A A C
    G T G T T C A G T T T A C C T T T G G A A G G T G A G T T G
                    790                 800                 810

      SER   SER   GLU   GLY   ASN  [LEU]  SER   PRO   SER   GLN
    C T C C T C C G A A G G C A A T C T A A G C C C T T C T C A
    G A G G A G G C T T C C G T T A G A T T C G G G A A G A G T
                    820                 830                 840

      VAL   SER   THR   THR   SER   GLU  [HIS]  PRO   SER   GLN
    A G T C T C C A C A A C A T C C G A G C A C C C A T C A C A
    T C A G A G G T G T T G T A G G C T C G T G G G T A G T G T
                    850                 860                 870

      PRO   SER   SER   PRO   PRO   ASN   THR  [THR]  ARG   GLN
    A C C C T C A T C T C C A C C C A A C A C A A C A C G C C A
    T G G G A G T A G A G G T G G G T T G T G T T G T G C G G T
                    880                 890                 900
```

***

```
GTAGTTATTAAAAAAAAAA
CATCAATAATTTTTTTTTTT
         910           920
```

NUCLEOTIDE SEQUENCE OF THE RSV G GENE. THE cDNA
SEQUENCE IS SHOWN IN THE PLUS (mRNA) STRAND
SENSE IN THE 5' TO 3' DIRECTION. THE TRANSMEMBRANE
(TM) ANCHOR DOMAIN IS UNDERLINED. AMINO ACIDS
DIFFERING FROM THE PUBLISHED PRIMARY SEQUENCE OF
THE PROTEIN ENCODED BY THE RSV G GENE ARE BOXED.

FIG.7D.

RESTRICTION MAP OF RSV G GENE

AflIII AhaII   SfanI BamHI PleI   NspV PstI   BgIII EarI   AspHI   BsrI

5'   250   500   750   3'

bp
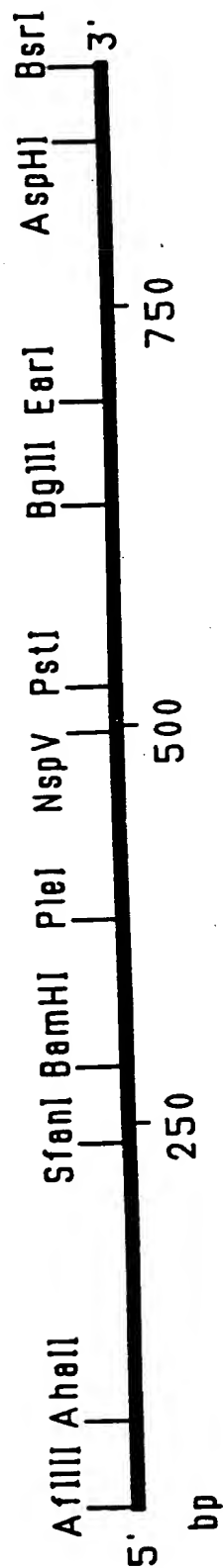
FIG.8.

Construction of a Bluescript-based expression vector containing the chimeric F_{PIV-3} -F_{RSV} gene with the 5' untranslated region of the PIV-3 F gene intact but lacking the nucleotide sequences coding for the hydrophobic anchor domains and cytoplasmic tails of both the PIV-3 and RSV F genes.

Step 1: Preparation of the plasmid containing the modified PIV-3 F gene

Cut with BamHI, blunt end and cut with Bsrl

Retrieve 1.6 Kb EcoRV-Bsrl PIV-3 F gene

Cut with EcoRV and BamHI

Ligate: 1.6 Kb [BamHI]-Bsrl F gene fragment + EcoRV-BamHI restricted vector +

```
         BspHI                          BamHI
  5'  __ATCAATCAAAGGTCCTGTGATAATAG____  3'
        CGTAGTTAGTTTCCAGGACACTATTATCCTAG
```

FIG.9A.

FIG.9B.   Step 2: Preparation of the plasmid containing the modified RSV F gene

T7 promoter

Kpnl
Apal
Dralll

Sspl

Neel

Afl oll   LacZ
Ap   MT promoter pMCR20
ORI   SV40polyA

Accl
Clal
HindIII
EcoRV
EcoRI
Pstl
Smal
BamHI

T3 promoter

Bglll

Xbal
Notl
Eagl
BstXl
SacII
Sacl

Cut with EcoRI and BamHI

Kpnl
EcoRV
EcoRI

BsplII

pRSVF RSV F
3'   5'

EcoRI
Smal
BamHI
Notl
Sacl

Cut with EcoRI and BspHI

Retrieve 1.6 Kb EcoRI-BspHI
RSV F gene fragment

Ligate: 1.6 Kb EcoRI-BspHI RSV F gene fragment +
EcoRI-BamHI restricted vector +

BspHI   BamHI
5' CATGACTTGATAATGAG.....3'

-----TGAACTATTACTCCTAG

T7 promoter

KpnI
ApaI
DraII

AccI
ClaI
HindIII
EcoRV
EcoRI

NaeI

Ap f1 orI LacZ
MT promoter pES13.A
ORI RSV F2+FI
SV40polyA

BspHI
BamHI

BglII

T3 promoter

Cut with EcoRI and PpuMI

EcoRI
LIGATE: 5' AATTCATGGAGTTGCTAATCCTCAAAGCAAATGCAATTAGCACAATCCTCACTGCAGTCACATTTTGTTTGCTTCTGGTTCTAAG ----
 ------GTACCTCAACGATTAGGAGTTTCGTTTACGTTAATGGTGTAATGGTGTTAGGAGTGACGTCAGTGTAAAACAAAACGAAGACCAAGATTCCAG
 PpuMI

T7 promoter

KpnI
ApaI
DraII

AccI
ClaI
HindIII
EcoRV
EcoRI

SspI

NaeI

Ap f1 orI LacZ
MT promoter pES23.A
ORI RSV FI LF2
SV40polyA

PpuMI

BspHI
BamHI

BglII

T3 promoter

FIG.9C.

Step 3 : Preparation of the plasmid containing the chimeric FpIV-3 -F RSV gene
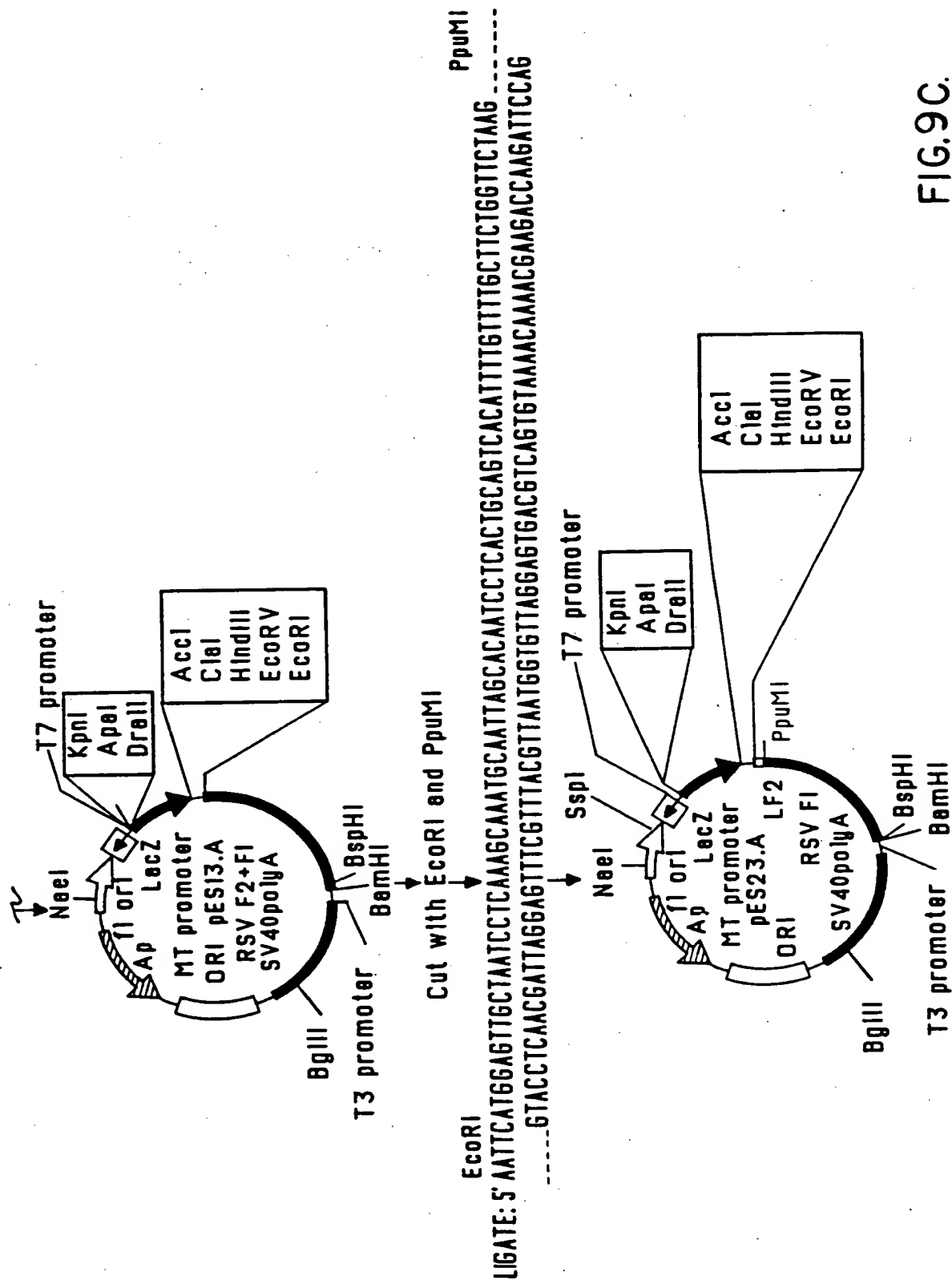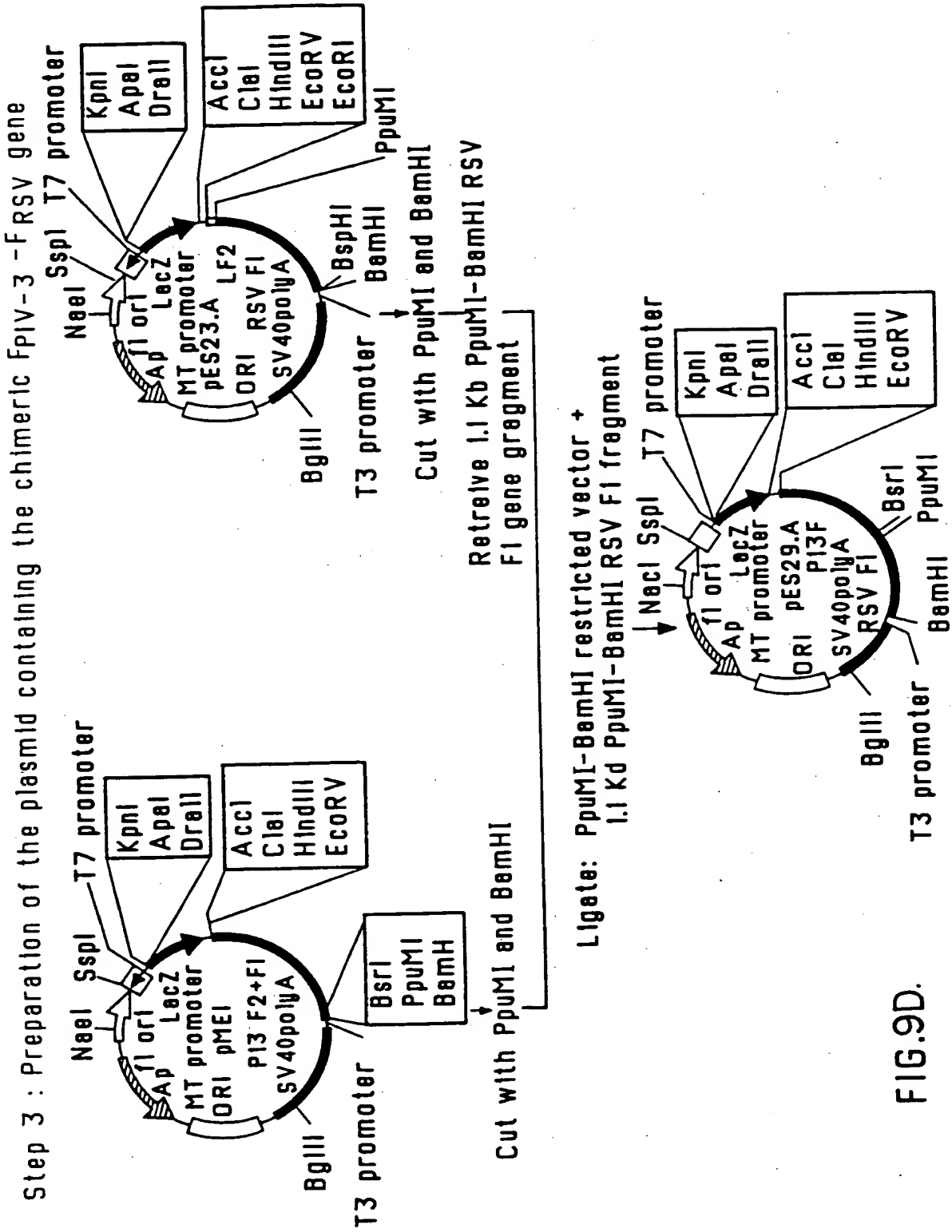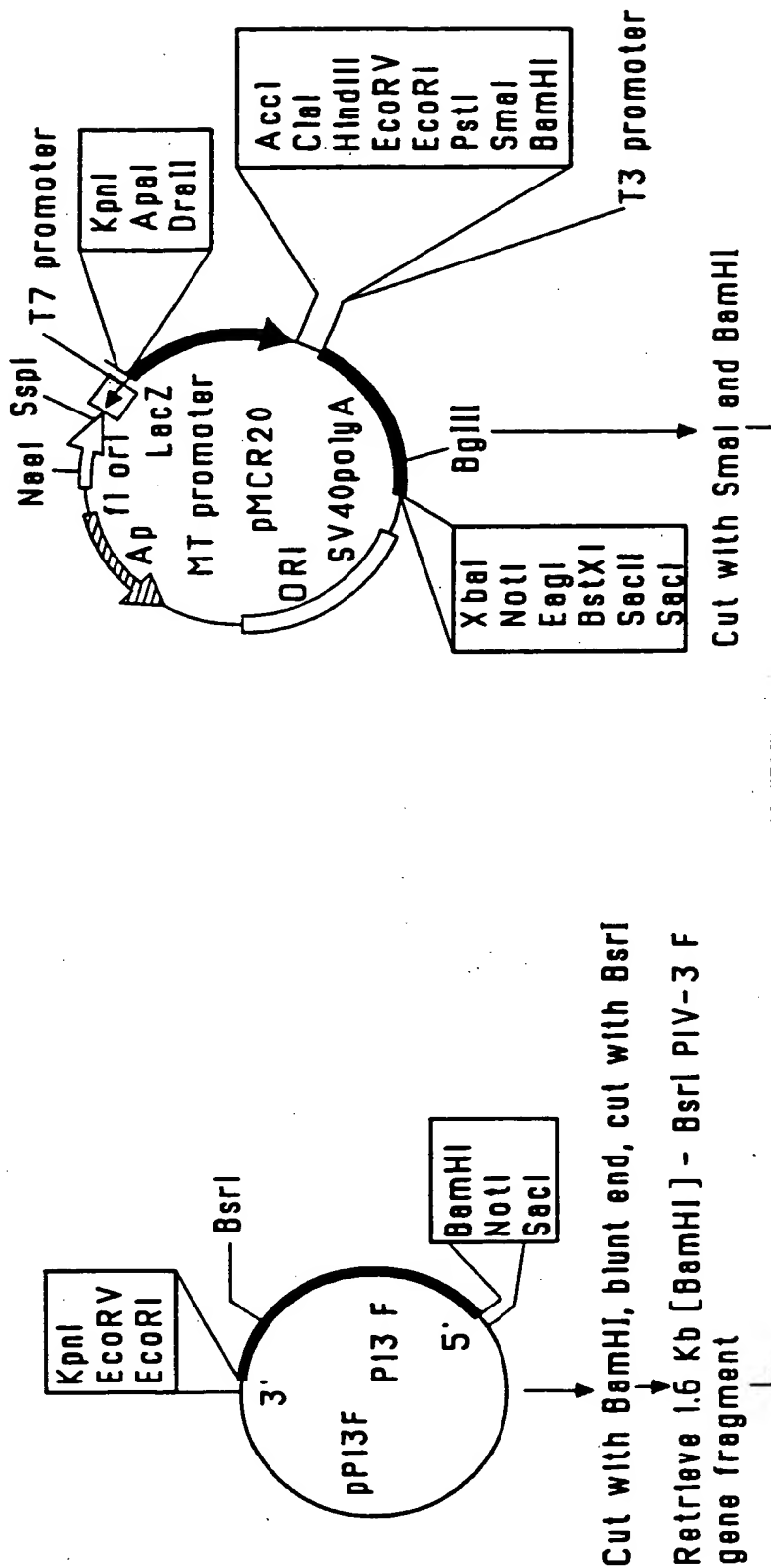
FIG.9D.

Construction of a Bluescript-based expression vector containing the PIV-3 F gene lacking the 5' untranslated sequence and transmembrane anchor and cytoplasmic tail coding regions.

Kpnl
Apal
Drall

Accl
Clal
HindIII
EcoRV
EcoRI
Pstl
Smal
BamHI

T7 promoter

T3 promoter

Nael Sspl

Ap fl ori
LacZ
MT promoter
pMCR20
ORI
SV40polyA

BglII

Xbal
Notl
Eagl
BstXI
SacII
Sacl

Cut with Smal and BamHI

Kpnl
EcoRV
EcoRI

Bsrl

BamHI
Notl
Sacl

3'   PI3 F   5'

pPI3F

Cut with BamHI, blunt end, cut with Bsrl

Retrieve 1.6 Kb [BamHI] - Bsrl PIV-3 F gene fragment
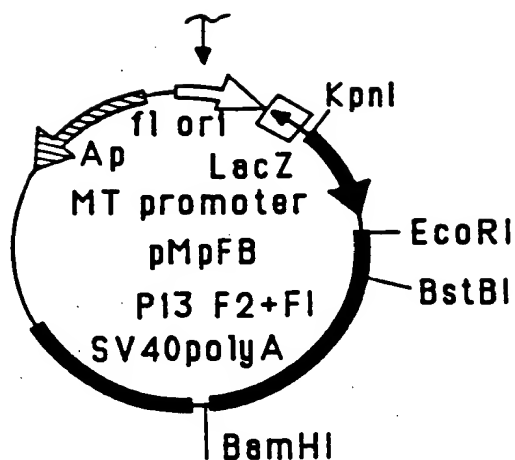
Ligate: 1.6 Kb [BamHI] - Bsrl PIV-3 F gene fragment +
Smal-BamHI restricted vector +

Bsrl   BamHI
5' ACTGGCATCAATCTAGCACTACACATGAG---- 3'
   ----CGTAGTTAGATCGTGATGTACTCCTAG

FIG.10A.

FIG.10B.

Cut with EcoRI and BstBI

Retreive: EcoRI-BstBI restricted vector

Ligate: EcoRI-BstBI restricted vector +

EcoRI   PpuMI

```
AATTCATGCCAACTTTAATACTGCTAATTATTACAACAATGATTATGG
CATCTTCCTGCCAAATAGATATCACAAAACTACAGCAATGTAGGTGTA
TTGGTCAACAGTCCCAAAGGGATGAAGATATCACAAAACTT____3'
____GTACGGTTGAAATTATGACGATTAATAATGTTGTTACTAATACC
GTAGAAGGACGGTTTATCTATAGTGTTTTGATGTCGTACATCCACATA
ACCAGTTGTCAGGGTTTCCCTACTTCTATAGTGTTTTGAAGCTT
```
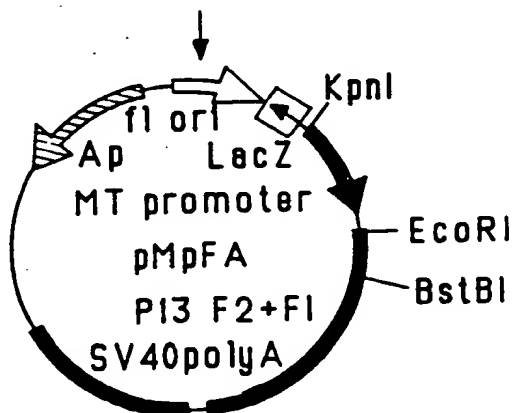
Construction of the chimeric Fpiv-3-FRSV gene consisting of the truncated
PIV-3 F gene devoid of the 5' untranslated region linked to the truncated RSV F1 gene.
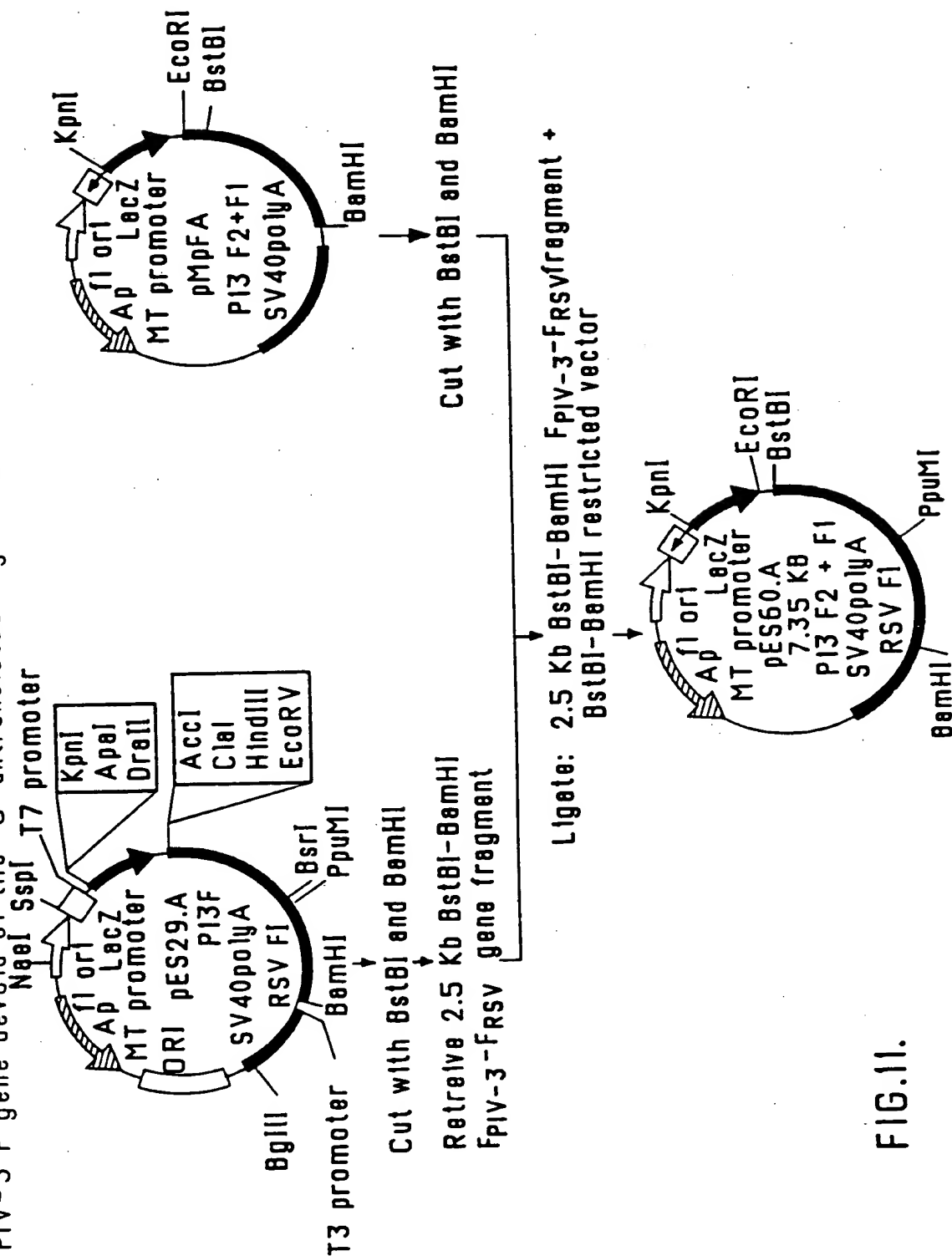
FIG.11.

FIG.12A. Construction of the modified pAc 610 baculovirus expression vector containing the chimeric $F_{PIV-3}-F_{RSV}$ gene consisting of the PIV-3 F gene lacking both the 5' untranslated sequence as well as the transmembrane and cytoplasmic tail coding regions linked to the truncated RSV F1 gene

Kpnl
Apal
Drall

Accl
Clal
HindIII
EcoRV
EcoRI

T7 promoter

Nael

Apfl orI
[acZ
MT promoter   pES13.A
ORI
RSV F2+F1
SV40polyA

BspHI
BamHI

BglII
T3 promoter

Cut with EcoRI and BamHI

Retreive 1.6 Kb EcoRI-BamHI
RSV F gene gragment

Hind III

pUC8
pAC 610

Eco RV

PstI.SalI.XbaI.BamHI.SmaI.SstI.EcoRI

Hind III

Cut with EcoRV and BamHI

Ligate: 1.6 Kb EcoRI-BamHI RSV F gene fragment +
        EcoRV-BamHI restricted vector +

EcoRV   EcoRI
5' ATCATGGAGATAATTAAAATGATAACCATCTCGCAAATAAATAAGTATTTTACTGTTTCGTAACAGTTTTGTAATAAAAAAACCTATAAATAG   3'
   TAGTACCTCTATTAATTTTACTATTGGTAGAGCGTTTATTTATTCATAAAATGACAAAAGCATTGTCAAAACATTATTTTTTGGATATTTATCTTAA

FIG.12B.

Top plasmid:

Kpnl
Apf1 ori
LacZ
MT promoter
pES60A
7.35 Kb
P13 F2+F1
SV40polyA
RSV F1
EcoRI
BstBI
PpuMI
BamHI

Cut with ECORI and BamHI

Retrieve 2.8 Kb EcoRI-BamHI
FPIV-3-FRSV gene fragment

Bottom-left plasmid:

HInd III
pUC 8
pES47A
Polyhedrin
promoter
ECO RV
ECO RI
PolyA
RSV F2+F1
HInd III
HInd III
BamHI

Cut with EcoRI and BamHI

Ligate: 2.8 Kb EcoRI-BamHI FPIV-3-FRSVfragment +
EcoRI-BamHI restricted vector

Bottom-right plasmid:

HInd III
pUC 8
pACDR7
Polyhedrin
promoter
P13 F2+F1
ECO RV
ECO RI
PolyA
RSV F1
HInd III
HInd III

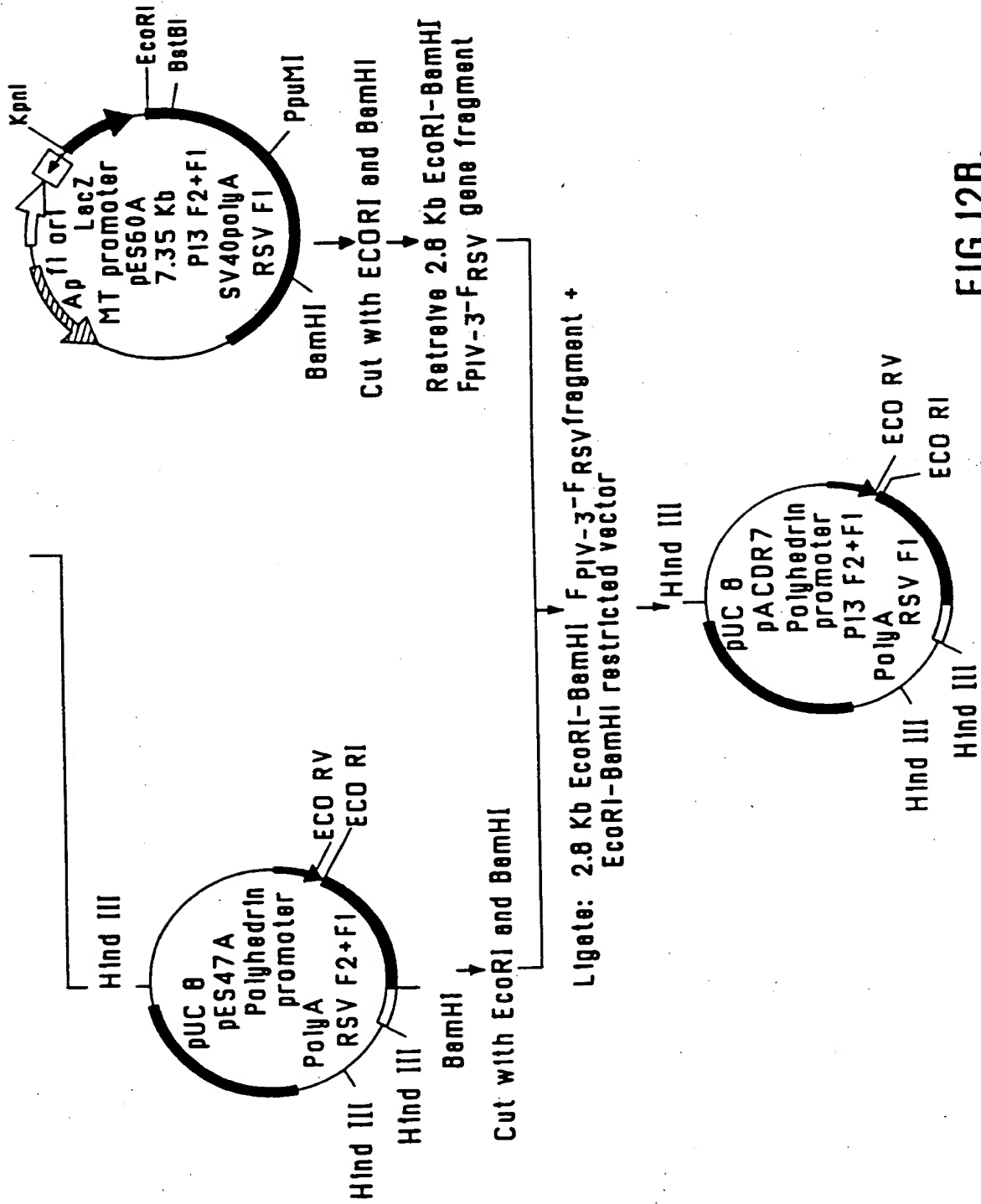# FIG.13
IMMUNOBLOTS OF CELL LYSATES FROM Sf9 CELLS INFECTED WITH RECOMBINANT BACULOVIRUSES
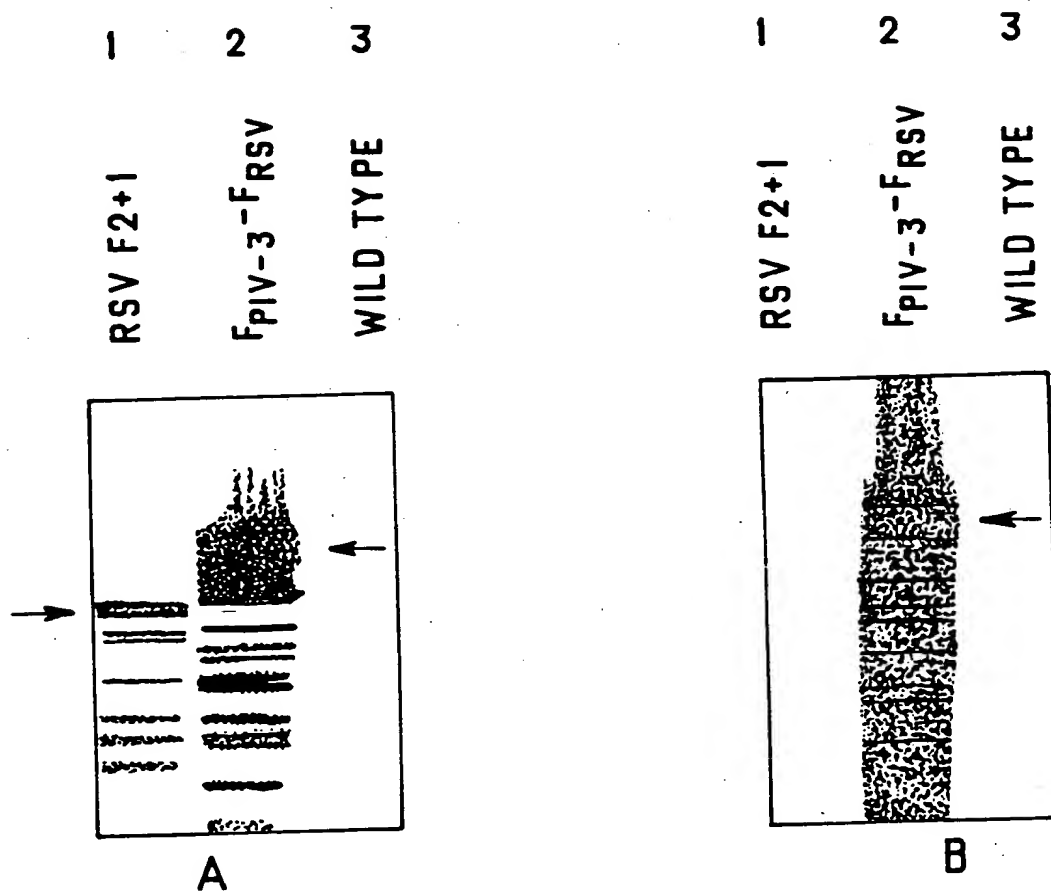

FIG 13 : Immunoblots of cell lysates from Sf9 cells infected wirth recombinant baculoviruses containing the truncated RSV F gene (Lane 1), the chimeric F_{PIV-3}-F RSVgene (Lane 2) or infected with wild type virus (Lane 3) reacted with anti-F RSV Mab (panel A) and anti-F1 PIV-3 antiserum (panel B)

FIG.14. CONSTRUCTION OF THE BACULOVIRUS TRANSFER VECTOR pD2

EcoRI
EcoRV / BamHI 0.04
HindIII

POLYHEDRIN
pVLI392
pUC8

HindIII

Cut with EcoRV and BamHI

EcoRV
5' ATCATGGAGATAATTAAAAATGATAACCATCTCGCAAATAATAAGTATTTACTGTTTCGTAACAGTTTGTAATAAAAAACCTATAAAT
TAGTACCTCTATTAATTTTACTATTGGTAGAGCGTTTATTTATTCATAAATGACAAAGCATTGTCAAAACATTATTTTTCGATATTTA
+1   +6 EcoRI   Polylinker
Polyhedrin promoter

                                                                    BamHI
ATTCCGGAATTCAGATCTGCAGCGGCCGCTCCATCTAGAAGGTACCCGG   3'
TAAGGCCTTAAGTCTAGACGTCGCCGGCGAGGTAGATCTTCCATGGGCCCTAG

Ligate

EcoRI
EcoRV / BamHI
HindIII

POLYHEDRIN
pD2
pUC8

HindIII

FIG.15 A. CONSTRUCTION OF THE F_{RSV}-HN_{PIV3} CHIMERIC GENE
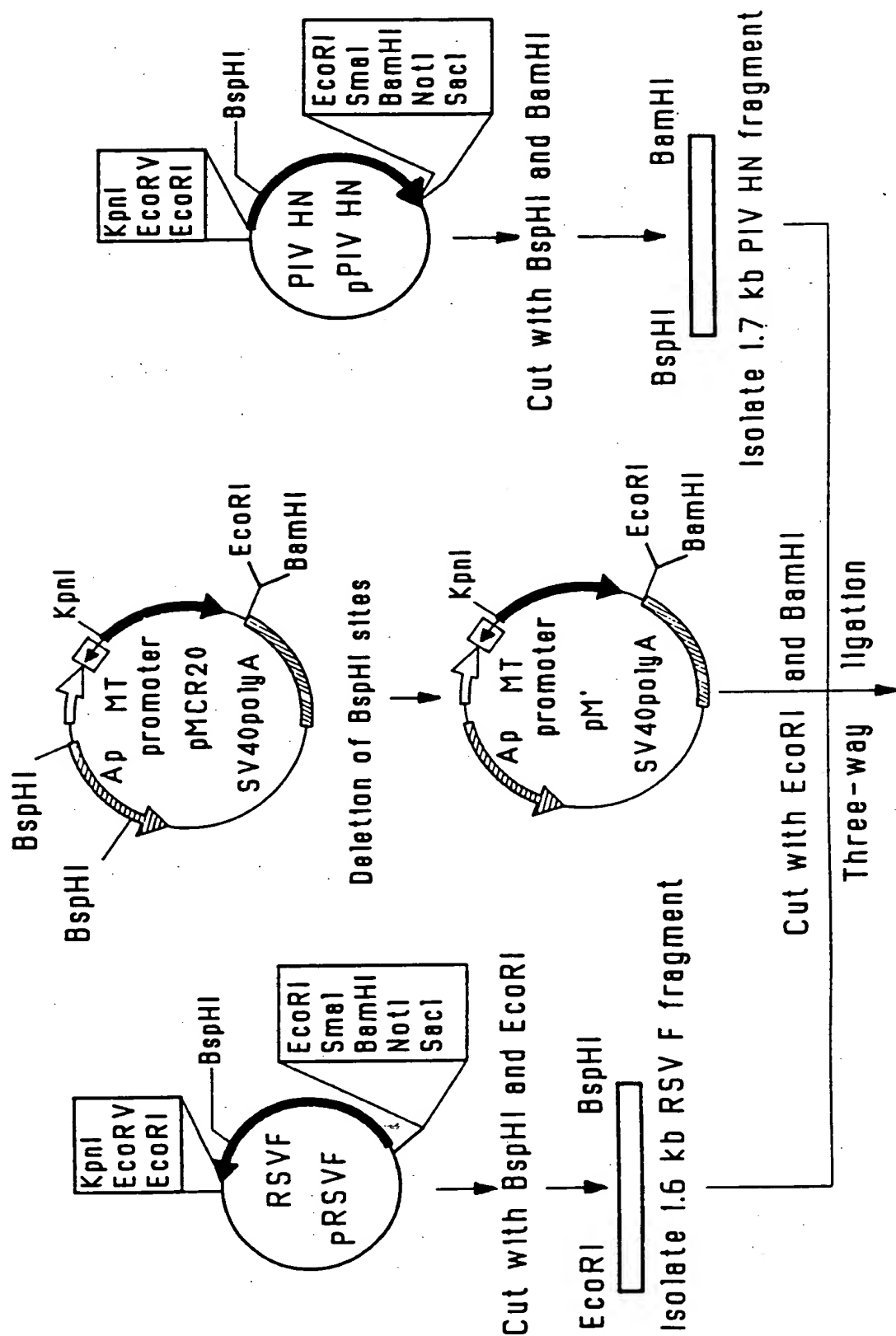
FIG.15B.

THREE-WAY ⅄LIGATION

↓

Kpnl

Ap MT
promoter
pM' RF-HN
poly A   RSVF

EcoRl

PIV HN

BspHI

BamHI
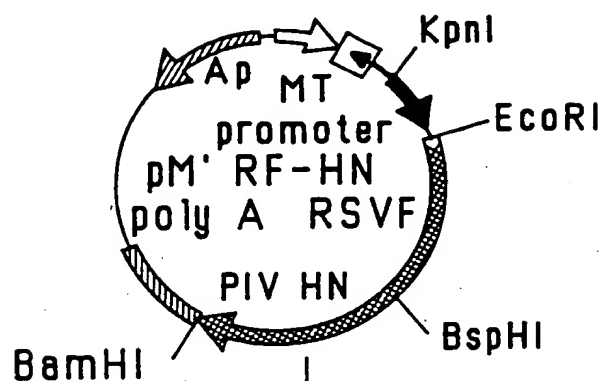
↓ Cut with BspHI

BspHI   BspHI
5' CATGACTAATTCCATCAAAAGTGAAAAGGCT   3'
         TGATTAAGGTAGTTTTCACTTTTCCGAGTAC

↓ Ligation of BspHI-BspHI linker

Kpnl

Ap MT
promoter
pM' RF-HN
poly A RSV F

EcoRl

PIV HN

BspHI

BamHI
Smal
EcoRl
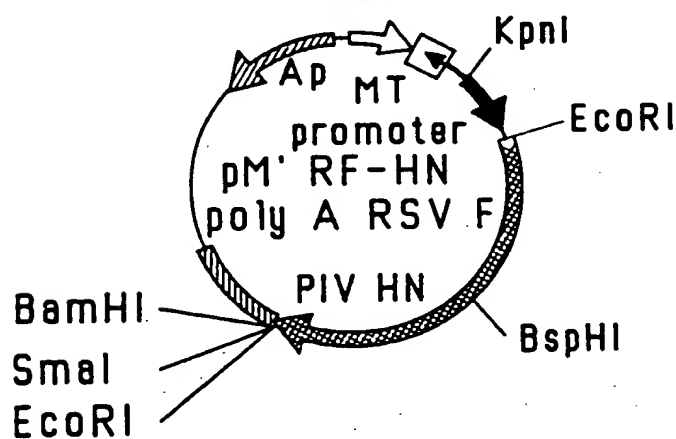
FIG.16

SDS POLY ACRYLAMIDE GEL AND IMMUNOBLOTS OF PURIFIED F_{RSV}-HN_{PIV-3} CHIMERIC PROTEIN

FIG 16 : A) Coomassie-stained SDS polyacrylamide gel of immunoaffinity- purified F_{RSV}-HN_{PIV-3} protein.

B) Immunoblots of F_{RSV}-HN_{PIV-3} protein reacted with an anti-F RSV Mab (lane 1) and anti-HN PIV-3 antiserum (lane 2)

FIG.17.   MUTAGENESIS OF THE PIV-3 F GENE

```
          ┌──────────┐
          │  KpnI    │
          │  EcoRV   │
          │  EcoRI   │
          └──────────┘
                │
                      ──── BsrAI
          ┌─────────────┐
         (   PI3FC      )
         (   pPI3FC     )
          └─────────────┘
                      ──── BsrI
                │
                   ┌──────────┐
                   │  EcoRI   │
                   │  NotI    │
                   │  SacI    │
                   └──────────┘
```
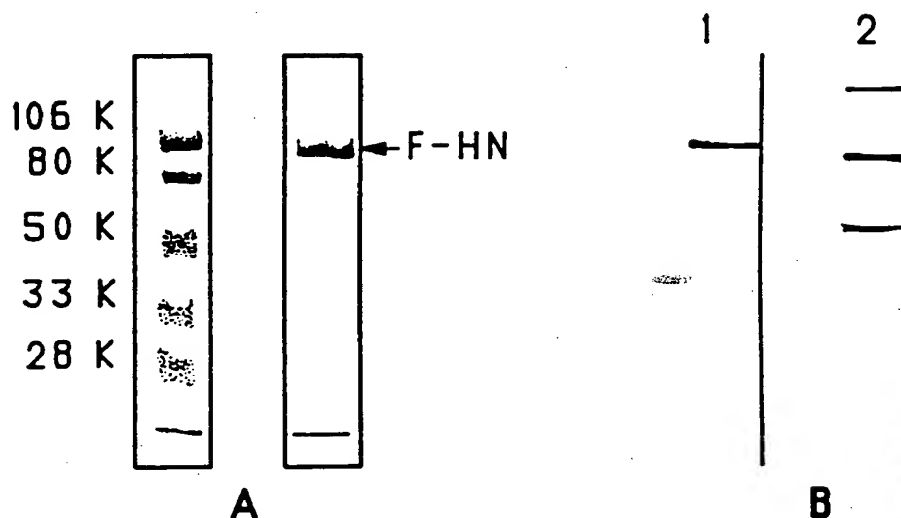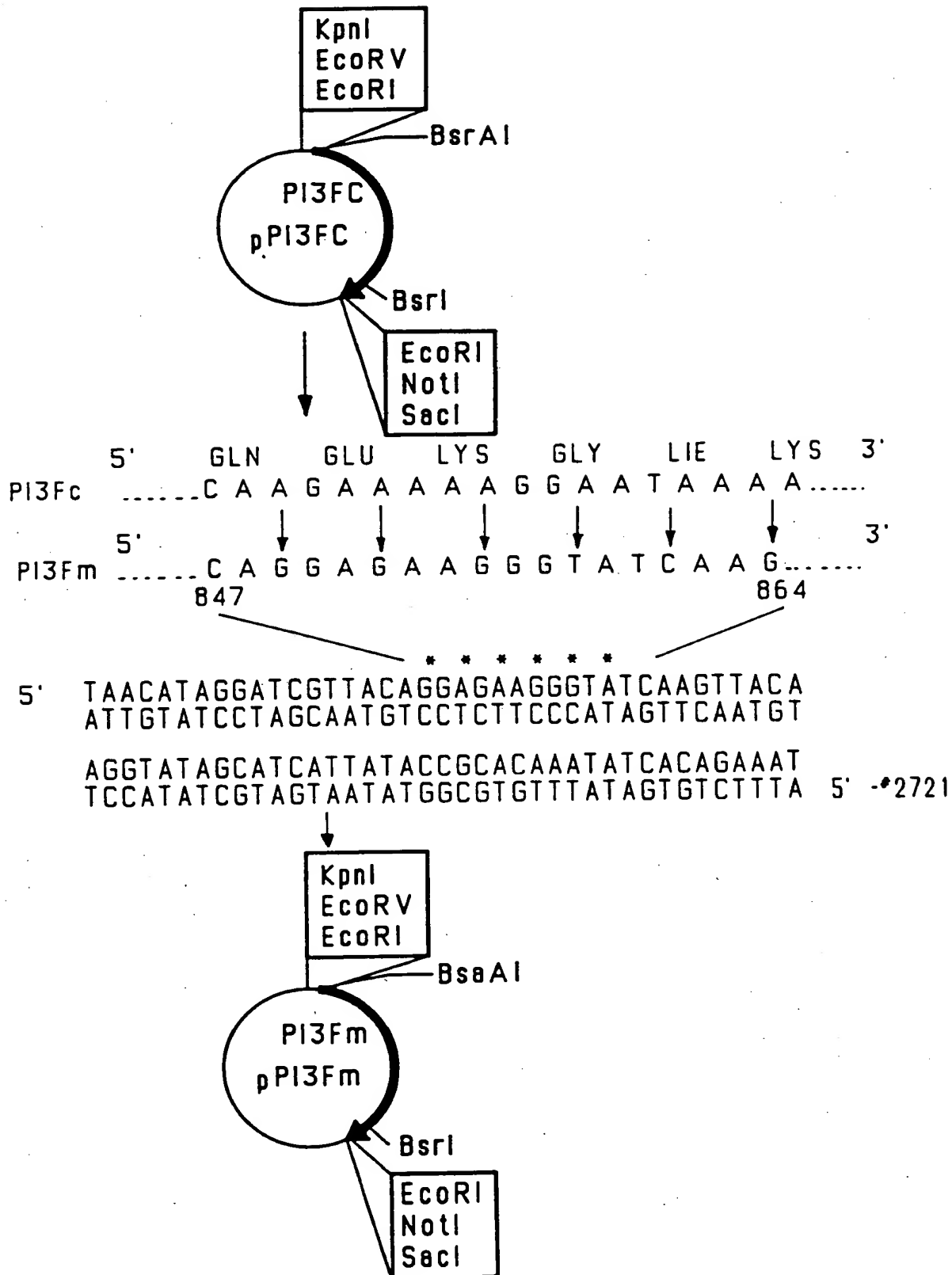
	5'	GLN	GLU	LYS	GLY	LIE	LYS 3'

PI3Fc   ......C A A G A A A A A G G A A T A A A A......

PI3Fm   ......C A G G A G A A G G G T A T C A A G......
       847   864

                    * * * * * *

5'   TAACATAGGATCGTTACAGGAGAAGGGTATCAAGTTACA
     ATTGTATCCTAGCAATGTCCTCTTCCCATAGTTCAATGT

     AGGTATAGCATCATTATACCGCACAAATATCACAGAAAT
     TCCATATCGTAGTAATATGGCGTGTTTATAGTGTCTTTA 5' -#2721

```
                   ┌──────────┐
                   │  KpnI    │
                   │  EcoRV   │
                   │  EcoRI   │
                   └──────────┘
                        │
                          ──── BseAI
                   ┌─────────────┐
                  (   PI3Fm      )
                  (   pPI3Fm     )
                   └─────────────┘
                          ──── BsrI
                        │
                       ┌──────────┐
                       │  EcoRI   │
                       │  NotI    │
                       │  SacI    │
                       └──────────┘
```

FIG.18. CONSTRUCTION OF THE F_{PIV3}-G_{RSV} CHIMERIC GENE

KpnI
EcoRI

Bsrl
BamHI
AflI (AGT)

RSV G
pRSVG

EcoRI
BamHI
NotI
SacI

Cut with EcoRI and BamHI

EcoRI   BamHI

0.95 Kb RSV Gt fragment

KpnI
EcoRI
BamHI
NotI
SacI

pPI3-SK

Cut with EcoRI and BamHI

KpnI
EcoRV
EcoRI

BsaAI

PIVFm
pPI3Fm

Bsrl

EcoRI
NotI
SacI

Cut with EcoRI
Religate

Two-way ligation

KpnI
EcoRI

Bsrl

BamHI
NotI
SacI

RSV Gt
pRSVGt

Cut with NotI
and BamHI

Bsrl   BamHI
5'ATCAATCTAGCACTACACAG   3'
CGTAGTTAGATCGTGATGTGTCCTAG

KpnI
EcoRV
EcoRI

Bsrl

PIVFmr
pPI3Fmr

BsaAI

EcoRI
NotI
SacI

Cut with Bsrl

Bsrl   NotI

1.7 Kb PIV Fmt fragment

Three-way ligation

KpnI
EcoRI

Bsrl
BamHI
Bsrl

RSV Gt
pFG
PIV Fmt

EcoRI
BamHI
NotI
SacI